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(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis to generate a synthesized DNA product; and

(c) inserting said synthesized DNA product into a TA cloning vector.

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32. (Amended) The method of claim 30, or 31, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

33. (Amended) The method of claim 23, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

34. The method of claim 23, 30 or 31, wherein said reaction mixture further comprises a PCR enhancing factor and/or an additive.

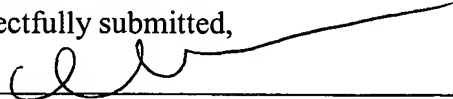
B6
35. (New) The method of claim 32, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

REMARKS

Claims 1-3, 6-14, 18, 20-26, 30-35 are currently pending. All pending claims are supported throughout the specification, for example, at least on pages 19-67. No new matter has been added by the foregoing amendments. A sheet showing the marked-up version of the amendments is attached.

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Respectfully submitted,


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Marked-up Version of Amendments

In the Claims

Please cancel claims 4-5, 9, 15-17, 19, 27-29 and replace claims 1-3, 6-14, 18, 20-26, 30-34 with the following claims. Please add new claim 35.

1. (Amended) An enzyme mixture comprising a first enzyme and a second enzyme, wherein said first enzyme comprises a DNA polymerization activity, and said second enzyme [comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity]is a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.
2. The enzyme mixture of claim 1, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.
3. The enzyme mixture of claim 2, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UI Tma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.
4. (Cancelled) The enzyme mixture of claim 1, wherein said second enzyme is a mutant DNA polymerase.
5. (Cancelled) The enzyme mixture of claim 4, wherein said mutant DNA polymerase is derived from a DNA polymerase different from said first enzyme.
6. (Amended) An enzyme mixture [for DNA synthesis]comprising a first enzyme and a second enzyme, wherein said first enzyme is a wild type Pfu DNA polymerase, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.
7. (Amended) An enzyme mixture for DNA synthesis comprising a first enzyme and a second enzyme, wherein said first enzyme is [a Taq]Archaeal DNA polymerase, said second enzyme is a mutant [Pfu]Archaeal DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.

8. (Amended) The enzyme mixture of claim [4]7, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: UITma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.
9. (Amended) The enzyme mixture of claim 6, [7, or 8,]wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.
10. (Amended) The enzyme mixture of claim 1 or 9, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.
11. (Amended) The enzyme mixture of claim [6, 7, or 8]1, further comprising a PCR enhancing factor and/or an additive.
12. (Amended) A kit comprising a first enzyme, [and]a second enzyme, and packaging material therefor, wherein said first enzyme comprises a DNA polymerization activity, said second enzyme [comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity, and packaging material therefore] is a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.
13. The kit of claim 12, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.
14. The kit of claim 13, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UITma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.
15. (Cancelled) The kit of claim 14, wherein said second enzyme is a mutant DNA polymerase.

16. (Cancelled) The kit of claim 15, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: ULTma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.
17. (Cancelled) The kit of claim 16, wherein said mutant DNA polymerase is derived from a DNA polymerase different from said first enzyme.
18. A kit comprising an enzyme mixture for DNA synthesis, said kit comprises a first enzyme and a second enzyme, and packaging material therefore, wherein said first enzyme is a wild type Pfu DNA polymerase, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.
19. (Cancelled) A kit comprising an enzyme mixture for DNA synthesis, said kit comprises a first enzyme and a second enzyme, and packaging material therefore, wherein said first enzyme is a Taq DNA polymerase, and packaging material therefore, said second enzyme is a mutant Pfu DNA polymerase comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity.
20. (Amended) The kit of claim 12, or 18, [or 19,]further comprising one or more components selected from the group consisting of: a deoxynucleotide, a reaction buffer, a PCR enhancing factor and/or an additive, a control DNA template and a control primer.
21. (Amended) The kit of claim [15,]18[, or 19,] wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.
22. (Amended) The kit of claim 12 or 21, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.
23. (Amended) A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a first enzyme comprising a DNA polymerization activity, and a second enzyme [comprising a 3'-5' exonuclease activity and a reduced DNA polymerization activity] which is a mutant Pfu DNA polymerase comprising one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

24. (Amended) The method of claim 23, wherein said nucleic acid template is a DNA [or an RNA] molecule.

25. The method of claim 24, wherein said first enzyme is a DNA polymerase or a reverse transcriptase.

26. The method of claim 25, wherein said DNA polymerase is selected from the group consisting of: Taq DNA polymerase, Tth DNA polymerase, UITma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

27. (Cancelled) The method of claim 24, wherein said second enzyme is a mutant DNA polymerase.

28. (Cancelled) The method of claim 27, wherein said mutant DNA polymerase is derived from a DNA polymerase selected from the group consisting of: UITma DNA polymerase, Tli DNA polymerase, Pfu DNA polymerase, KOD DNA polymerase, JDF-3 DNA polymerase, PGB-D DNA polymerase and DP1/DP2 DNA polymerase.

29. (Cancelled) The method of claim 27, wherein said mutant DNA polymerase is derived from a DNA polymerase different from said first enzyme.

30. A method for DNA synthesis comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a wild type Pfu DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a

second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity; and

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis.

31. A method for TA cloning of DNA synthesis product comprising:

(a) providing an enzyme mixture, said enzyme mixture comprising a Taq DNA polymerase as a first enzyme, and a mutant Pfu DNA polymerase as a second enzyme which comprises a 3'-5' exonuclease activity and a reduced DNA polymerization activity;

(b) contacting said enzyme mixture with a nucleic acid template, wherein said enzyme mixture permits DNA synthesis to generate a synthesized DNA product; and

(c) inserting said synthesized DNA product into a TA cloning vector.

32. (Amended) The method of claim [28,]30, or 31, wherein said mutant Pfu DNA polymerase comprises one or more mutations at amino acid positions selected from the group consisting of: D405, Y410, T542, D543, K593, Y595, Y385, G387, and G388.

33. (Amended) The method of claim 23 [32], wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.

34. The method of claim 23, 30 or 31, wherein said reaction mixture further comprises a PCR enhancing factor and/or an additive.

35. (New) The method of claim 32, wherein said mutant Pfu DNA polymerase comprises one or more mutations selected from the group consisting of: D405E, Y410F, T542P, D543G, K593T, Y595S, Y385Q, Y385S, Y385N, Y385L, Y385H, G387S, G387P, and G388P.